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Lombard effect onset times reveal the speed of vocal plasticity in a songbird

Running title: Lombard effect onset times in a songbird

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Keywords: Lombard effect, bird song, amplitude, noise, bioacoustics, response time

Summary statement: We measured the speed at which canaries adjust the amplitude of their songs to mitigate the effects of signal masking by noise and demonstrate rapid vocal plasticity in this species.

Abstract

Animals that use acoustic signals to communicate often compensate for interference and masking from background noise by raising the amplitude of their vocalisations. This response has been termed the Lombard effect. However, despite more than a century of research little is known how quickly animals can adjust the amplitude of their vocalisations after the onset of noise. The ability to respond quickly to increases in noise levels would allow animals to avoid signal masking and ensure their calls continue to be heard, even if they are interrupted by sudden bursts of high amplitude noise. We tested how quickly singing male canaries (*Serinus canaria*) exhibit the Lombard effect by exposing them to short playbacks of white noise and measuring the speed of their responses. We show that canaries exhibit the Lombard effect approximately 300 ms after the onset of noise and are also able to increase the amplitude of their songs mid-song and mid-phrase without pausing. Our results demonstrate high vocal plasticity in this species and suggest that birds are able to adjust the amplitude of their vocalisations very rapidly to ensure they can still be heard even during sudden changes in background noise levels.

Introduction

Acoustic communication is often constrained by the masking effects of background noise which can prevent a signal from being heard by the targeted receiver. To overcome this problem animals may adjust their acoustic signals in a variety of different ways including increasing the duration of brief calls (Brumm et al. 2004), increasing the redundancy of their vocalisations by giving longer and more repetitive call series (Brumm & Slater, 2006; Kaiser & Hammers, 2009), shifting the timing of their vocalisations (Fuller et al. 2007; Vargas-Salinas & Amézquita, 2013),

52 or by increasing the pitch of their vocalisations (Slabbekoorn & Peet, 2003; Parks et al. 2007).
53 One of the most efficient and widespread methods by which animals reduce the impact of signal
54 masking is by raising the amplitude of their vocalisations (Brumm & Zollinger, 2011; Hotchkin
55 & Parks, 2013). This phenomenon has been termed the Lombard effect in honour of its
56 discoverer, the French otolaryngologist Etienne Lombard (Zollinger & Brumm 2011), and it has
57 been shown to be much more effective at increasing signal detectability in noise than either
58 increasing the duration or repetition of a vocalisation (Luo et al. 2015). Moreover, even increases
59 in the pitch of vocalisations in response to noise may in fact be a by-product of calling more
60 loudly, as higher pitched sounds can generally be produced at higher amplitudes (Nemeth et al.
61 2013).

62 The Lombard effect has now been shown across a diverse range of taxa including
63 mammals, such as humans, monkeys, cetaceans, and bats (Hotchkin & Parks, 2013) and
64 numerous species of paleognath and neognath birds (Brumm & Zollinger, 2011). The situation
65 in amphibians is still unresolved as one study recently found a noise-dependent regulation of
66 call amplitudes in a frog, (Halfwerk et al. 2015) whereas previous studies failed to find evidence
67 for the Lombard effect in other anuran species (Schwartz & Bee, 2013). Recently, the presence
68 of the Lombard effect was also reported in a fish (Holt & Johnston, 2014) but the data is difficult
69 to interpret because it is not clear whether and how the noise amplitudes were accounted for in
70 the signal measurements in this study.

71 The widespread taxonomic distribution of the Lombard effect suggests it is the basic
72 mechanism by which birds and mammals, and perhaps also other vertebrates, improve the
73 detectability of their vocalisations in noise. Furthermore, in birds the Lombard effect occurs
74 independently of whether vocalisations are learnt through vocal production learning (Cynx et
75 al. 1998; Brumm & Todt, 2002) or not (Potash, 1972; Leonard & Horn, 2005; Schuster et al. 2012).
76 In humans however, it has been shown that the strength of the Lombard effect can be affected

77 by the social context (Amazi & Garber, 1982; Lu & Cooke, 2008) or linguistic content of the
78 vocalisation (Patel & Schell, 2008), and may also be voluntarily controlled to some extent by
79 cognitive processes (Pick et al. 1989; Tonkinson, 1994).

80 Although evidence for the Lombard effect in animals is extensive, it has so far only been
81 demonstrated in either wild animals living in continuously noisy environments, or in captive
82 animals exposed to long periods of synthetic noise (Tab. 1). Early studies of the Lombard effect
83 often used the term "Lombard reflex" (e.g. Egan, 1971; Junqua, 1996), possibly hinting that the
84 Lombard effect is typically exhibited very quickly in response to noise. This was shown in
85 humans by Bauer et al. (2006) who found an onset latency of 157 ms when the amplitude of the
86 auditory feedback of a speaker's own voice was increased via headphones. Foery (2008) found a
87 similar onset latency of 127 ms in humans exposed to playbacks of noise.

88 However, despite this and more than a century of research on the subject, only one
89 study has yet directly tested how quickly the Lombard effect can be triggered in a non-human
90 animal. Hage et al. (2013) found that greater horseshoe bats (*Rhinolophus ferrumequinum*) with
91 their highly specialized auditory orientation system may adjust the amplitude of their
92 echolocation calls as fast as approximately 150 milliseconds, but data on other taxa, and
93 especially on vocalizations used for communication, are still lacking. This omission from the
94 literature is surprising given that many animals are often exposed to sudden changes in noise
95 levels. In undeveloped natural habitats, falling branches, gusts of wind or the calls of con- and
96 heterospecifics are potential sources of intermittent high amplitude noise bursts (Luther &
97 Gentry, 2013). In urban areas peaks in traffic during the morning and evening rush hours
98 contribute to a daily fluctuation in noise levels, while short bursts of often very loud noise from
99 sources such as car alarms, car horns, construction work or passing vehicles are common
100 throughout the day (Warren et al. 2006; Luther & Gentry, 2013).

The ability to rapidly increase the amplitude of their vocalisations would allow animals to avoid signal masking and ensure their calls continue to be heard, even if their vocalisations are interrupted by very sudden bursts of high amplitude noise. This is likely to be particularly important for species whose vocalisations encode information as complex sequences of different elements since masking of any part of these signals may prevent the correct messages from reaching their targeted receivers. For species whose signals encode warnings about threats or predators (e.g. Seyfarth et al. 1980; Templeton et al. 2005) it may be even more important to ensure that they are successfully transmitted. As animal vocalisations are also often used for territory defence and mate attraction (Seyfarth & Cheney, 2003), intermittent masking of these vocalisations may lead to reduced breeding success in some species. The ability to avoid signal masking during sudden bursts of noise is therefore likely to be strongly favoured by selection.

On a proximate level, the Lombard effect demonstrates that the auditory system continuously monitors vocal output and uses this feedback to modulate the sound pressure level of vocalisations according to the strength of the background noise (Eliades & Wang, 2012). The speed at which the Lombard effect can act is therefore dependent on the neurons of the auditory system, and the speed with which the muscles of the sound producing organ can respond to neuronal stimulation (Suthers & Zollinger, 2008). In mammals the neurons which mediate the Lombard effect are located in the brainstem (Nonaka et al. 1997; Hage et al. 2006) and in the auditory cortex (Eliades & Wang, 2012). The ability of humans to voluntarily control the Lombard effect to some degree also points towards some involvement of the motor cortex (Brumm & Zollinger, 2011). These studies suggest that control of the Lombard effect is sophisticated and likely requires well-developed auditory and motor control systems.

Understanding how quickly animals are able to exhibit the Lombard effect will provide insight into how these systems function together and may also advance our understanding of the flexibility and plasticity of vocal behaviours in animals.

We addressed this topic in a small passerine bird, the canary (*Serinus canaria*: Linnaeus). Male canaries have diverse and well-studied vocal repertoires that are used in mate attraction and stimulation (e.g. Leitner et al. 2001; Voigt & Leitner, 2008; Leboucher et al. 2012). The use of minibreaths during rapid trills, which allows for uninterrupted songs of very long duration and consisting of long repetitive phrases of the same element type, makes the canary an ideal model to investigate mechanisms of rapid song modulation (Suthers et al. 2012). Furthermore, different aspects of song production and vocal control mechanisms have been studied in this species (e.g. Leitner & Catchpole, 2004; Bolhuis & Gahr, 2006; Suthers et al. 2012), which will eventually allow placing new findings on the mechanisms of vocal plasticity into a broader behavioural physiology context (Elemans et al. 2015). We examined changes in the sound pressure level of the song of canaries during sudden short and sporadic bursts of broadband white noise in order to discover how rapidly they exhibit the Lombard effect.

138 **Table one.** Duration of noise exposure in studies of the Lombard effect in non-human animals.

Species	Context	Duration of noise exposure (sound pressure level re. 20 µPa unless stated otherwise)	Reference
Birds			
Japanese quail (*Coturnix coturnix japonica*)	Captive	Three hours prior to recordings (48 – 63 dB)	Potash (1972)
Zebra finch (*Taeniopygia guttata*)	Captive	Until a minimum of five vocalisations had been produced (60 – 90 dB in 5 dB increments)	Cynx et al. (1998)
Budgerigar (*Melopsittacus undulatus*)	Captive	Until the bird had produced 75 calls. During the first 25 calls noise was played at 55 dB followed by 70 dB for the second 25 calls and 55 dB again for the last 25 calls.	Manabe et al. (1998)
Common nightingale (*Luscinia megarhynchos*)	Captive	20 minutes or until the bird had sung 27 songs, repeated at 5 dB noise increments between 55 – 75 dB	Brumm & Todt (2002)
Bengalese finch (*Lonchura striata domestica*)	Captive	100 seconds per noise treatment played successively at 40 – 70 dB at 10 dB increments.	Kobayasi & Okanoya (2003)
Blue-throated hummingbird (*Lampornis clemenciae*)	Wild	Until the bird stopped producing chipping calls (35 and 40 dB)	Pytte et al. (2003)
Common nightingale (*Luscinia megarhynchos*)	Wild	Continuous urban noise (40 – 64 dB)	Brumm (2004)
Tree swallow (*Tachycineta bicolor*)	Captive and wild	Wild birds – continuous ambient noise (41 – 67 dB) Captive birds – At least one hour (55 and 65 dB)	Leonard & Horn (2005)
Domestic fowl (*Gallus gallus*)	Captive	Six minutes per treatment with four successive noise treatments (60, 67, 75 and 80 dB)	Brumm et al. (2009)
Budgerigar (*Melopsittacus undulatus*)	Captive	Until the bird had produced 60 vocalisations (40 – 90 dB)	Osmanski & Dooling (2009)
Noisy miner (*Manorina melanocephala*)	Wild	Continuous urban noise (50.83 – 65.80 dB)	Lowry et al. (2012)
Elegant crested tinamou (*Eudromia elegans*)	Captive	Until the bird had called 12 times (45 and 65 dB in 5 dB(a) increments)	Schuster et al. (2012)
Amphibians			
Cope's grey treefrog (*Hyla chrysoscelis*)	Captive	Six minutes (40, 50, 60, 70 dB), Lombard absent in this species	Love & Bee (2010)
Túngara frog (*Physalaemus pustulosus*)	Captive	One minute (54 – 94 dB)	Halfwerk et al. (2015)
Mammals			
Crab-eating macaque (*Macaca fascicularis*), and southern pig-tailed macaque (*Macaca nemestrina*)	Captive	Until 10 vocalisation had been produced at each of five playback levels in ascending and descending intensity (70, 80, 90, 80, 70 dB)	Sinnott et al. (1975)
Common marmoset (*Callithrix jacchus*)	Captive	30 minutes per noise treatment played successively in a random order at 40, 50, 60 and 65 dB.	Brumm et al. (2004)
Cotton-top tamarin (*Saguinus oedipus*)	Captive	Until 11 calls in both 50 and 70 dB noise had been produced	Egnor & Hauser (2006)
North Atlantic right whales (*Eubalaena glacialis*)	Wild	Continuous exposure to environmental noise (92 – 143 dB re: 1µPa)	Parks et al. (2011)
Mexican free-tailed bat (*Tadarida brasiliensis*)	Captive	A minimum of 100 echolocation pulses (55, 65, 75, 85 dB)	Tressler & Smotherman (2009)
Killer whale (*Orcinus orca*)	Wild	Continuous noise exposure from passing ships (approx- 96 – 118 dB re. 1µPa)	Holt et al. (2009)
Greater horseshoe bat (*Rhinolophus ferrumequinum*)	Captive	30 seconds (80, 90, 100 dB)	Hage et al. (2013)
Pale spear-nosed bat (*Phyllostomus discolor*)	Captive	28, 40, 52 dB (six minutes)	Luo et al. (2015)
Fish			
Blacktail shiner (*Cyprinella venusta*)	Captive	17 minutes to 2.5 hours. Playback noise equivalent to ambient noise levels in nesting sites and 10.2 dB louder than the quiet treatment (re. 1µPa)	Holt & Johnston (2014)

Methods

Animals

Recordings were taken from seven adult male canaries (*Serinus canaria*: Linnaeus) kept under license (license number: Az.: 311.5-5682.1/1-2014-021). One female canary was used to encourage the males to sing. All birds were bred and raised in aviaries at the Max Planck Institute for Ornithology (Seewiesen, Germany). Experiments were performed under a 14:10 light:dark cycle, and constant access to *ad libitum* food and water supplemented with fresh vegetables, cuttlebones and grit.

Apparatus

Prior to experiments, all male birds were kept together in an aviary (1.95 × 1.0 × 1.8 m). The female was kept in a separate cage (120 × 80 × 60 cm). Recordings were made in a separate aviary (1.95 × 1.0 × 1.8 m) lined with acoustic tiles which was visually and audibly separated from the other birds. During recordings, individual males were placed inside the recording aviary inside a wire cage (60 × 40 × 40 cm) within view of the female (in a separate cage 2 m away). An omni-directional microphone (Sennheiser ME62) connected to a PC using an external soundcard (Edirol UA-101) and the recording software Sound Analysis Pro (Tchernichovski et al. 2000; version 1.085) were used for all audio recordings. Recordings were made with a sampling rate of 44.1 kHz and 16 bit accuracy. The microphone was placed 60 cm above the centre of the cage, halfway between the two perches, to minimise variation in the recorded sound level caused by lateral movements of the bird's body and head. Recordings were triggered automatically whenever the bird sang using the trigger-record function in Sound Analysis Pro.

To induce the Lombard effect, white noise (0.1 – 16 kHz) was played towards the birds during singing bouts. The noise was broadcast through a JBL Pro III loudspeaker placed 140 cm away from the cage and connected to a Pioneer A109 stereo amplifier. The sound pressure level of the noise was 75 dB (re. 20µPa) measured from inside the cage at the position of the perches.

A custom Matlab (version 7.5.0; Natick, USA; www.mathworks.com) routine was used to automatically trigger noise playbacks whenever the sound pressure level and duration of a bird's song crossed a pre-defined trigger threshold. The trigger function was controlled using a microphone (Audio-Technica ATR3350) connected to an external soundcard (Edirol UA25) and the Playrec toolkit for Matlab. Canary song is composed of a succession of phrases which are each formed from long repeats of different song elements (Poulsen, 1959; Nottebohm & Nottebohm, 1978). We set white noise playbacks to begin after a random delay of between 1 - 10 seconds after being triggered to ensure that the noise began during a different phrase and element type in each recording. Playbacks of white noise were always exactly 20 seconds long.

Analysis

All acoustic analyses were carried out using the software Avisoft-SASLab Pro (version 5.2.09; Specht, 2002). First, different song element types were identified using spectrograms and each song element type was given a number unique to the element type itself and to the bird it came from. The sound pressure level for each element in both noise and quiet conditions was measured with an averaging time of 10 ms.

The sound pressure level of the background noise was subtracted from these measurements using the following logarithmic computation procedure given by Brumm & Zollinger (2011) in order to calculate the sound pressure level of the song elements alone (Lsignal):

$$\text{Eqn 1. } L\text{signal} = 10\log(10(L\text{signal} + \text{noise}/10) - 10(L\text{noise}/10))$$

Where Lsignal + noise is the sound pressure level of the song element and the background noise and Lnoise is the sound pressure level of the background noise alone.

The software was calibrated by recording a sine tone of constant amplitude using the same microphone and software settings as used for the recordings of birdsong. The sound

pressure level of this tone (68 dB, 1000 Hz) was directly measured using a sound pressure level meter (Voltcraft SL-400) at the position of the microphone.

The proportional increase in the sound pressure level (SPL) of song elements in noise was calculated using the equation:

$$\text{Eqn 2. SPL increase} = \sqrt{2}\text{^}(\text{dB increase}/6 \times 2)$$

Where *SPL increase* is the proportional increase in the sound pressure level and *dB increase* is the measured increase in dB of a song element.

For every song element sung during noise playbacks we also recorded how many seconds of the noise playback the bird had been exposed to before the element was sung (maximum 20 seconds). With this information we created a subset from our full dataset for song elements sung during the first one second after the onset of noise exposure. These data were used to determine if canaries exhibit the Lombard effect within one second of exposure to noise.

In most of our recordings canaries stopped singing immediately after the noise playback began, before quickly resuming song again in the noise. However, in some cases the canaries continued to sing the same phrase uninterrupted during the quiet period and into the noise (Fig. 1). From these recordings we created a separate dataset of sound pressure level measurements to test if canaries can adjust the sound pressure level of their songs mid-song and mid-phrase without pausing.

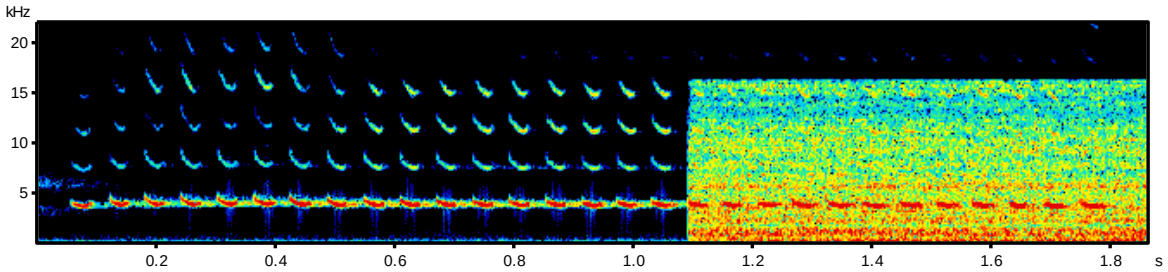


Figure 1. Spectrogram showing canary song which began during quiet conditions and continued after noise began.

Statistical analyses

To determine if the Lombard effect occurred within 20 seconds and within one second of the onset of noise, and to determine whether canaries are able to exhibit the Lombard effect mid-phrase without first interrupting their song, we analysed our data using generalised linear mixed models (GLMMs) in R (version 3.0.2; R core team, 2013) using the package lme4. We modelled the influence of noise exposure (binary fixed factor: present or absent) on the sound pressure level of the song elements (dependent variable) with normally distributed errors and an identity link function. The ID of the birds, the song element code and the recording (the audio file the data were taken from) were all included as random factors. We also included noise as a nested random factor within recordings to account for audio files containing song elements recorded both during quiet and during noise exposure. We assessed the effect of noise exposure on the sound pressure level of canary song by comparing models including noise exposure to null models using likelihood ratio tests with one degree of freedom. Where multiple analyses were carried out on the same dataset Bonferroni corrections were used to account for multiple comparisons.

To more precisely determine the speed of the onset of the Lombard effect we further analysed one exemplary element type from one individual (for which the most data were

228 available) using a broken-line regression model fitted using the segmented package in R
229 (Muggeo, 2008; Muggeo & Adelfio 2010). This allowed us to precisely identify at what time after
230 the onset of noise this element type was sung at a significantly higher sound pressure level than
231 before the noise began.

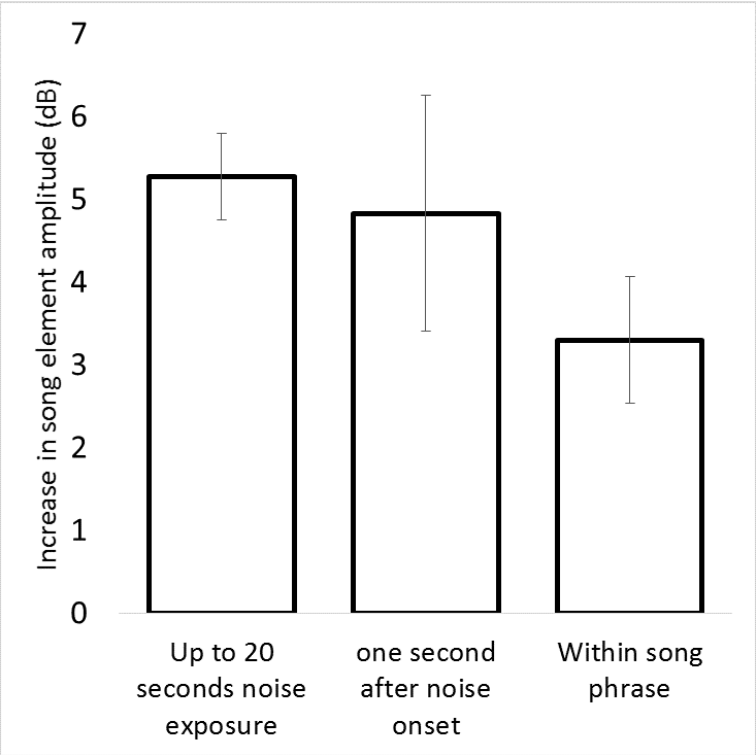
232 ## Results

233 In total we measured 4140 song elements from seven birds (n = 1750 before the onset of noise,
234 2390 during white noise exposure, song element types n = 31). The sound pressure level of song
235 elements sung during the full 20 seconds of exposure to white noise was on average 5.27 dB (±
236 0.52 s.e.m.) higher than song elements sung before the onset of noise (χ^2 = 60.166, d.f. =
237 1, Bonferroni corrected P < 0.0001), while the sound pressure level of song elements sung during
238 the first one second of noise exposure (n = 636 before the onset of noise, 38 during white noise
239 exposure, birds n = 3, song element types n = 5) was on average 4.83 dB (± 1.42 s.e.m.) higher
240 than song elements sung before the onset of noise (χ^2 = 9.430, d.f. = 1, Bonferroni corrected P =
241 0.004). These results represent increases in sound pressure level of 84 % and 75 % respectively
242 (Fig. 2).

243 In many of our recordings we found that canaries often briefly stopped singing
244 immediately after the noise playback began. However, in some of our recordings the birds began
245 to sing a song phrase during quiet conditions and continued to sing the same phrase
246 uninterrupted as a noise playback began (Fig. 1; n = 159 elements sung before the onset of noise,
247 80 during white noise exposure, birds n = 4, mean phrase length 1.5 s). These recordings allowed
248 us to test if canaries are able to exhibit the Lombard effect mid-song and mid-phrase without
249 pausing. In these recordings, the sound pressure level of song elements sung during noise
250 exposure was on average 3.3 dB (± 0.76 s.e.m.) higher than song elements sung before the onset
251 of noise (χ^2 = 8,940, d.f. = 1, P = 0.002) representing a 46 % increase in the sound pressure level
252 (Fig. 2).

253 For the element type analysed using the broken-line regression model we found that the
254 sound pressure level of song elements sung more than 0.318 seconds after the onset of noise was
255 significantly higher (P = <0.05) than song elements sung before the onset of noise (Fig. 3).

256



257

258 **Figure 2. Mean (± s.e.m.) increases in the sound pressure level of song elements sung**
259 **during noise compared to the period before the onset of noise.** Graph shows the increase
260 in the sound pressure level of song elements sung up to 20 seconds after the onset of noise
261 exposure (n = 1750 before the onset of noise, 2390 during white noise exposure, song element
262 types n = 31), during the first one second of noise exposure (n = 636 before the onset of noise, 38
263 during white noise exposure, birds n = 3, song element types n = 5) and within song phrases
264 which began during the quiet period and continued uninterrupted into the noise (n = 159
265 elements sung before the onset of noise, 80 during white noise exposure, birds n = 4). All bars
266 show a significant increase in the sound pressure level of elements sung after the onset of noise
267 when (P = <0.05).

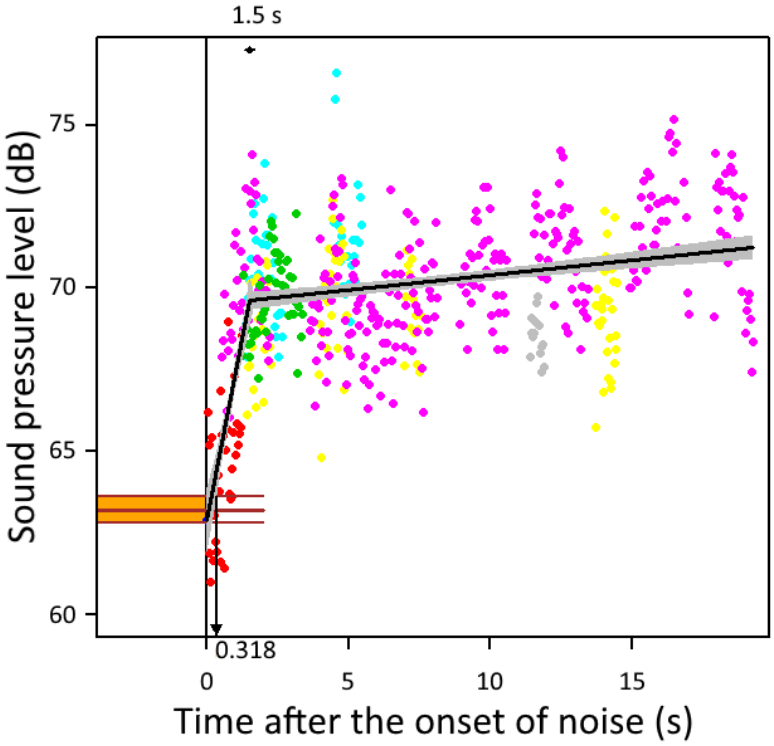


Figure 3. Scatterplot showing the sound pressure level of individual song elements before and after the onset of noise. Shown is one element type taken from a single bird for which the most data were available (n = 46 elements measured before the onset of noise; 207 during white noise). Each point depicts a single vocalisation and different colours show measurements taken from different recordings. The mean sound pressure level of song elements sung in the five seconds before the onset of noise is depicted by the brown line with orange bands showing the 95 % confidence intervals. The black line was fitted using a broken-line regression model with grey bands showing the 95 % confidence intervals. The onset of the Lombard effect is defined as the point at which the 95 % confidence intervals for elements sung before and after noise no longer overlap and occurred here at 0.318 seconds after the onset of noise. Elements sung after this time point were significantly louder (P = <0.05) than elements sung before the noise began. The initial rapid increase in the sound pressure level of song elements stopped at 1.5 seconds as shown at the top of the panel. After this point the increase in the sound pressure level slowed markedly.

Discussion

This is the first study, to our knowledge, to directly test the onset latency of the Lombard effect in a bird and the first to show that the Lombard effect is exhibited by canaries. Our results show that male canaries exhibit the Lombard approximately 300 ms after the onset of noise, and are able to do this mid-song and mid-phrase without pausing. Thus, we show that canaries possess a remarkably fast vocal plasticity which allows them to adjust their vocalisations in real time to mitigate the masking effects of sudden bursts of noise. Given that the Lombard effect is an ancient trait which is likely shared by all extant bird species (Brumm & Zollinger, 2011) it is probable that all vocalising species exhibit similarly rapid response times. Moreover, while humans exhibit the Lombard effect within 150 ms (Bauer et al. 2006; Kristen R. A. Foery, Triggering the Lombard Effect: Examining Automatic Thresholds, master's thesis, University of Colorado at Boulder, 2008), greater horseshoe bats (*Rhinolophus ferrumequinum*) have been shown to increase the sound pressure level of their echolocation calls almost instantaneously when exposed to noise (Hage et al. 2013), These studies suggest that similarly rapid Lombard responses may be present in taxa other than birds.

In recent years numerous studies have identified adjustments to the vocalisations of animals living in noisy environments which help them to mitigate the problem of signal masking (Brumm, 2013). Most of these studies contrast the vocalisations of populations living in noisy and quiet environments and identify differences which may be adaptive. However, it is still not fully understood exactly how these differences arise. Several hypotheses have been proposed, including short term vocal plasticity, long-term ontogenetic vocal adjustments, selective attrition of vocalisations which transmit poorly in noise, passive acquisition of vocalisations which transmit well in noise, and microevolutionary change (Patricelli & Blickley, 2006). The Lombard effect is a clear example of short-term vocal plasticity. The very fast reaction times demonstrated in our study suggests, in combination with the evidence for the

perceptual efficiency of the Lombard effect for signal detection in noise (Nemeth & Brumm 2010, Luo et al. 2015), that vocal plasticity is the key factor allowing animals to cope with the problem of signal masking in environments with unpredictable and fluctuating noise levels.

Studies on the speed of behavioural song plasticity in birds are rare but the available evidence suggest that other song parameters can be modified in response to changes in the environment on different or similar time scales as the one we found for the Lombard effect. House finches (*Carpodacus mexicanus*) have been shown to shift the frequency of their song notes within the time it takes them to sing three songs to avoid masking by high-amplitude noise playbacks (Bermúdez-Cuamatzin et al. 2010), while black-capped chickadees (*Poecile attricapillus*) increase the frequency of their song notes on average after more than one minute to avoid spectral overlap with lower frequency masking tones played back to them (Goodwin & Podos, 2013). In contrast, the onset of singing activity in nightingales (*Luscinia megarhynchos*) can be triggered on average within 0.9 seconds to reduce temporal overlap by heterospecific songs (Brumm, 2006). In duetting bird species, the two partners of a pair may react even faster to integrate their duet parts into one coherent song (Hall, 2009; Tempelton et al. 2013). Thus, the regulation of vocal onset in birds operates on a similar time scale as the Lombard effect, suggesting a similar role for the fast adjustment of signalling in fluctuating environments.

The rapid onset of the Lombard effect also indicates how quickly the auditory system can be integrated with the different motor systems to enable fast vocal plasticity (Bauer et al. 2006). For the Lombard effect to occur, a singing bird first needs to detect an increase in noise and in a second step increase the contraction of abdominal and intercostal muscles to increase bronchial pressure, which eventually leads to an increase in song amplitude (Plummer & Goller, 2008). To stay on pitch during Lombard song, birds need to decouple amplitude from frequency during vocal production, which could be achieved by a reduction of labial tension via the

syringeal muscles or a reduction of air pressure in the interclavicular air sac via the respiratory muscles (Elemans et al. 2015).

Our study also sheds light on the question of what the smallest unit of vocal production in birds is. Cynx (1990) approached this question by interrupting the song of zebra finches (*Taeniopygia guttata*) and observing at what point in their songs the birds stopped singing. He found that zebra finches always stopped singing at discrete locations between song elements and never stopped halfway through a song element. That song elements in zebra finches may represent the smallest motor unit of song production was further supported by the discovery of Yu & Margoliash (1996) of precisely timed temporal correlations between discharge patterns in the vocal motor nucleus RA (Robust nucleus of the archopallium) and individual song elements in singing birds. Franz & Goller (2002) later confirmed this result in the peripheral vocal production system, by showing that each song element sung by zebra finches corresponds to a single expiratory pressure pulse from the lungs. Evidence from measurements of peripheral vocal motor patterns in canaries also support the hypothesis that individual elements represent the smallest units of song production in this species. Even in trills with a repetition rate of up to 30 elements per second, canaries take a rapid minibreath between each element, demonstrating that each element in these trills represents a discrete production unit (Suthers & Zollinger, 2008). However, unlike in zebra finches (Yu and Margoliash, 1996), almost nothing is known about the smallest motor units of song production in the canary brain. In our study canaries often interrupted their song almost immediately in response to the sudden onset of noise. As in zebra finches, we observed that canaries always stopped singing at discrete intervals between elements. Our recordings therefore suggest that song elements are also the smallest units of sound production in this species. Furthermore, as repeats of individual syllables in canary song are controlled by the HVC and the song pattern by the RA (Halle et al. 2003), the ability to stop singing so quickly after the onset of noise suggests extremely rapid modulation of this pathway.

In conclusion, our study adds to the growing number of studies which show that animals use the Lombard effect to communicate in to noise. Furthermore, we show for the first time that the Lombard effect can be exhibited extremely rapidly in response to sudden bursts of noise. For animals that live in environments with highly variable and unpredictable background noise this ability is likely to be of particular importance as it would allow them to maintain signal transmission despite sudden changes in noise levels.

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